A STUDY OF VARIOUS PHASES OF DEVELOPMENT IN REPRESENTATIVE SPECIES OF THE GENUS *SCHIZOSACCHAROMYCES*

BY

JOSEPH FRANK PAGANO

B.S., University of Illinois, 1942

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SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BACTERIOLOGY IN THE GRADUATE COLLEGE OF THE UNIVERSITY OF ILLINOIS, 1949

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Introduction

The importance of yeast in commerce and science necessitates a more fundamental approach to an understanding of their behavior. Recent genetic studies on this group of organisms has supplied this approach. They show that yeasts are similar to higher plants in their genetic behavior, i.e., segregation of genes, mating types, alternation of generations, mutations, variations and inbreeding generation. Understanding the behavior of the chromosomal apparatus during the mitotic and meiotic divisions can largely explain the morphological and physiological activities of the yeast culture.

The studies which follow are an application of genetic analysis to single spore cultures of several species of the genus *Schizosaccharomyces.*

Historical

Yeast cells contain a nucleus whose exact structure is very much in dispute. During budding and cell fission the nucleus divides mitotically. Since the mitotic figures are difficult to demonstrate, the mechanism of division during vegetative reproduction is unsettled. Though chromosomes undoubtedly exist, their exact number and shape are unknown. Some cytological evidence exists that the chromosome number is reduced during sporulation. Segregation and hybridization studies substantiate meiosis as a regular process in the life cycle of spore forming yeasts. Additional proof of the affinity of the yeast nucleus to that of higher plants is obtained from its Feulgen reaction. In both cases a positive reaction is obtained due to the presence of thymonucleic acid.

Though cellular fusion in yeast had been observed by numerous investigators, the true significance of this process has only recently become evident (Winge 1935). As in the higher plants, the life cycle involves an alternation of generations. It has been

shown by single ascospore cultures that haploid gametes of *Saccharomyces* fuse to establish the diplophase. The diploid zygotes sporulate after a period of vegetative reproduction. Reduction division occurs resulting in spore nuclei having the haploid number of chromosomes. Various types of segregations occur during this meiosis (Winge 1944) analogous to the process found in higher plants. Both morphological and physiological segregations of simple and complex types have been demonstrated in yeast.

In a homozygous strain of *Saccharomyces cerevisiae* marked decrease in spore viability was observed which was attributed to inbreeding. Direct diploidization of the mother-daughter nuclei at germination of the ascospore was not accompanied by chondriosome division. The cells resulting from such diploidization were considered permanently deficient in chondriosomes.

The existence of mating types in yeast was observed by Lindegren (1943) in the genus *Saccharomyces.* Generally, it is recognized that yeast show a degree of heterothallism but probably the majority of them are homothallic.

One of the most promising aspects of yeast genetics is the breeding of inter-specific or intra-specific hybrids. The fate of genes entering into a hybrid cross can be followed and their segregation pattern determined.

Method

Three species of the genus *Schizosaccharomyces* were used in these investigations, i.e., *Schizosaccharomyces versatilis, Schizosaccharomyces octosporus,* and *Schizosaccharomyces japonicus.* Single spore cultures were made with the aid of a Zeiss micromanipulator. Photomicrographs were made of the various stages in the life cycle of *Schizosaccharomyces versatilis.*

Discussion of the Results

The germination of a single spore of *Schizosaccharomyces versatilits* was demonstrated by a series of photomicrographs. The first cells germinating from the single spores were elongated and devoid of granulation. Fusion of closely related cells takes place just prior to sporulation; this process transpires in small aggregates of cells.

Sporulation of *Schizosaccharomyces versatilis* and *Schizosaccharomyces japonicus* was abundant on solid medium containing 5% dextrose, and extremely rare when the medium contained 1% dextrose. *Schizosaccharomyces octosporus* sporulated well on solid medium but not in liquid medium, irrespective of dextrose concentration.

Liberation of ascospores, i.e., dehiscence, occurred in *Schizosaccharomyces versatilis* and *Schizosaccharomyces japonicus,* shortly after the formation of the ascus. Masses of liberated spores float on the surface of liquid medium. It was established that the ascospores contained within the ascus were of low germinating ability as compared to the free or floating spores found in these cultures. A period of maturation for the spores occurs after liberation from the ascus. Dehiscence was not prominent in *Schizosaccharomyces octosporus.*

In certain cases, concentration of asci was effected by a sugar floatation method using a 50% sucrose solution. Concentration was necessary in order to affect microdissection of asci of *Schizosaccharomyces versatilis.*

Single spore cultures isolated from the three species of *Schizosaccharomyces* were homothallic. Fusion takes place between closely related cells resulting in the sexual fruiting body, the ascus. There was no indication of mating types. The cells which fused were not differentiated either phenotypically or genotypically. Single spore cultures were examined for morphological and physiological segregation of genes. In no instance were the single spores isolated different from each other or from the original parent in fermentation reaction or giant colony formation. Apparently, our strains of *Schizosaccharomyces* are completely homothallic and homozygous. The effect of inbreeding in these homozygous cultures was not reflected in a significant decrease in production or viability of ascospores.

Summary

Single spore cultures isolated from *Schizosaccharomyces versatilis, Schizosaccharomyces japonicus* and *Schizosaccharomyces octosporus* were homothallic and homozygous,

The life cycle of *Schizosaccharomyces versatilis* was recorded by means of photomicrographs; fusion occurs between closely related sister-cells, just prior to ascospore formation.

Spore fertility was not markedly decreased by inbreeding in these homozygous cultures.

Sporulation of *Schizosaccharomyces versatilis* and *Schizosaccharomyces japonicus* cultures were affected by increased dextrose concentration (5% or 10%). The germination of single spores dissected from the asci of these two species was extremely low.

A rapid method of making single spore isolations or ascus dissections is discussed.

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VITA

The author was born July 19, 1919, in Cleveland, Ohio. He was graduated from Evander Childs High School (N.Y.) in 1938. He completed his undergraduate work at the University of Illinois with a major in Bacteriology in 1942 and received a Bachelor of Science degree. In August, 1942, he joined the U. S. Naval Reserve. He was honorably released to inactive duty in November, 1945. He returned to the University of Illinois for graduate work in Bacteriology, receiving a Master of Science degree in June, 1947. During this period he was employed as research assistant by the Illinois State Water Survey. In September, 1948, he was awarded a third year graduate Wright Fellowship. Having finished the experimental work and requirements for the Doctor's degree in February, 1949, the author accepted a position as Research Associate in the Department of Chemistry at the University of Illinois. The author is a member of Sigma Xi, Society of Illinois Bacteriologists and the Society of American Bacteriologists.

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